The Effects of an Intermittent Stimulus of Gravity on the Geotactic Behavior of the Land Snail *Helix aspersa* Müller

(Mollusca : Gastropoda : Pulmonata)

BY

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(2 Text figures)

The concepts of 'phototropism' and 'geotropism' originated early in the 19th century from the descriptions of directed movements of plants and unicellular organisms by De Candolle. Loeb (1916) employed the concept of tropisms in an attempt to describe the behavior of animals in the terms of the physical scientist. He applied two principles of photochemistry to the reactions of animals to light. The first of these, the Bunsen-Roscoe Law, states that the effect of light in a photosensitive reaction is a product of the intensity of the light and its duration. The second, Talbot's Law, deals with intermittent stimulation by light. It states that the effect of intermittent light is identical to that of continuous stimulation if the total amounts of light energy emitted are identical. Reciprocal variations of intensity and time of exposure will elicit a constant response.

Extensive investigation has been carried out to establish the validity of these principles. The major researchers include Loeb (1916, 1918), Northrop & Loeb (1923), Arnold & Winsor (1934), Wulf, Fry & Linde (1955), Alpern & Faris (1956), and Brindley (1956). All of the work to date has been concerned with photostimulation.

The purpose of this investigation was to determine whether or not Talbot's Law can be expanded to include gravitational stimuli.

The pulmonate land snail *Helix aspersa* Müller was subjected to vertical oscillations, which varied in speed and intensity, while on a variable-tilt platform. A snail is an ideal subject for this type of investigation because of its sensitive geonegative response and its ability to adhere to the substrate under conditions of intermittent gravity stimulation.

Vertical oscillations passing through a certain point average over time to an acceleration equivalent to that of one gravity. That *Helix* is capable of detecting and responding to an increased gravitational stimulation has been established by Hoagland & Crozier (1931) and by Cole (1926). Thus, if the present oscillated subjects respond to the varying gravitational forces in a manner not significantly differing from others on a stationary platform, the physiological effect of intermittently applied gravitational forces must be summated over time, and Talbot's Law can be expanded to include gravitational stimuli.

Extensions of the Bunsen-Roscoe Law have been shown to be valid for gravity by Britton *et al.* (1950) in their work on the effects of extreme increases and decreases in the total amount of gravitational stimulation on circulatory parameters in rats and monkeys.

Other workers at San Francisco State College are currently investigating stimulus parameters such as thermotaxis and galvanotaxis, in an attempt to formulate a general hypothesis concerning intermittent stimulation.

Helix aspersa used in this experiment were collected between April 22 and May 4, 1963, and were tested within four hours of the time of collection. They were stored in jars and cans which were uncovered 15 minutes prior to the experiment. Moist paper towelling was kept in the containers to insure maximum activity of the animals.

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The experimental apparatus consisted of a sheet of plywood, 48" by 22½" by ½", standing vertically on the floor. Two strips of ¼" aluminum tubing were set vertically on the face of the plywood, 5 inches apart and parallel to each other, and a sliding mechanism was constructed to run along the tubing (Figure 1). A 7" by 3" plastic-coated insect tray was mounted bottom-side up on the slider by a single bolt; the tray could be fixed at any angle to the horizontal plane.

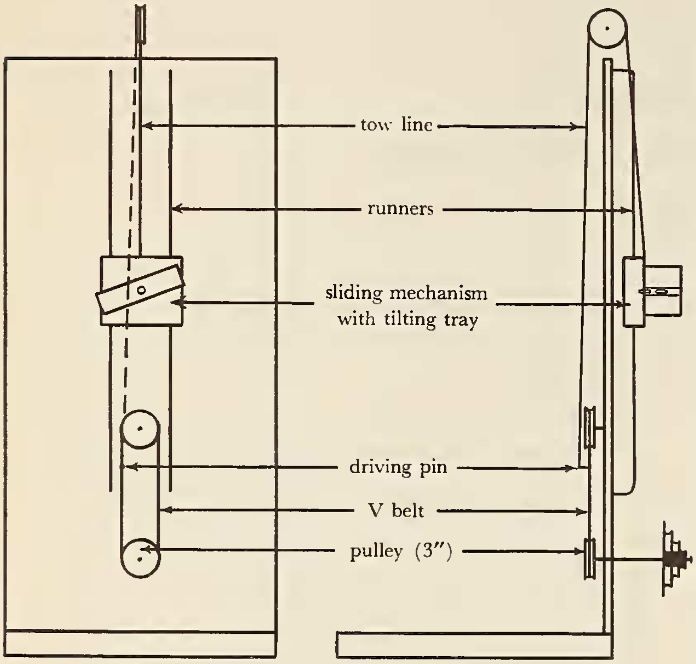

Figure 1:

Experimental Apparatus

The slider and tray were oscillated vertically by a mechanism powered by a ⅙ HP motor rated at 1725 rpm. A series of pulleys and V-belts was used to reduce the frequency of oscillation and to allow a four-way variation of speed at the output of the system. A three-stepped pulley was mounted on the motor drive shaft to deliver three speeds at the input of the system, giving a total of 12 speeds available at the output.

Transmission to the sliding mechanism was accomplished by inserting the output shaft through the face of the vertical plywood and mounting it on a 3" pulley. A second 3" pulley was mounted on an idling shaft directly above the first, and a 32" belt was rotated vertically. A pin attached to this belt was connected to the sliding mechanism by a line which was strung over a bearing pulley at the top of the plywood. As the belt rotated, it lifted and lowered the sliding mechanism along the aluminum runners at a speed selected in the driving mechanism. Speeds were available between 6 and 101 rpm, and the vertical distance traveled by the tray was 69 centimeters.

The tray was lined with a sheet of slick bond paper with two parallel lines across its width, each one inch from the center. A circle ½ inch in diameter was drawn between them to serve as the starting circle. The liner was replaced after every trial to prevent any snail from following the mucous trail of a preceding animal. The room was darkened, and the apparatus covered with a cardboard carton during each test. The tray was set at the selected experimental angle, and an active experimental animal was placed in the starting circle, facing away from the attachment of the tray and slider. The box was then closed and the oscillation begun. After three minutes the oscillation was stopped, the door opened, and the animal's position on the tray noted. If the anterior edge of the shell had passed the line on the elevated side of the starting circle its position was noted as "up", if it had passed the line on the lowered side of the starting circle its position was noted as "down". Animals which had come in contact with the sliding mechanism which was not inclined at the experimental angle, and those that had not moved at all, were not counted.

A different group of 20 snails was tested at each of the four experimental angles (2, 8, 14, and 20 degrees) without oscillation as a control. Groups of 20 snails were then tested at five different frequencies of intermittent acceleration at each experimental angle (9.5, 14.5, 26, 41, and 54 rpm). A total of 480 snails were tested under the described conditions.

In order to determine actual changes in acceleration on the tray, the apparatus was placed in a dark room and covered with black cloth. A white strip was placed on the sliding mechanism. A strobe-light, which had been found accurate by calibration against Welch tuning forks, was used to deliver light flashes of known frequency. The mechanism was operated at test speeds and a multiple exposure was made with a Polaroid camera. This instrument was rotated through a horizontal plane to avoid superimposition of images and one entire cycle was recorded. The positive prints were then projected on white paper with an opaque projector. The images of the white strip were traced and measured, yielding time and distance values, from which the average rate of change

Table 1

Orientation of *Helix aspersa* in six treatments at each of four angles, with the values of χ-square and associated probabilities for upward orientation at each of the angles, and the measured range of gravitational force (in gravities) for each oscillation rate.

		revolutions per minute										
Angle	Direction	0	9.5	14.5	26	41	54	Total	X	S	χ^2	P
2°	Up	8	12	10	11	12	11	64				
	Down	12	8	10	9	8	9	56				
	Total	20	20	20	20	20	20	120	10.7	1.1	1.32	0.93
8°	Up	14	15	13	16	15	15	88				
	Down	6	5	7	4	5	5	32				
	Total	20	20	20	20	20	20	120	14.7	1.3	1.26	0.94
14°	Up	18	16	18	17	17	19	105				
	Down	2	4	2	3	3	1	15				
	Total	20	20	20	20	20	20	120	17.5	1.1	2.25	0.82
20°	Up	20	20	20	20	20	20	120				
	Down	0	0	0	0	0	0	0				
	Total	20	20	20	20	20	20	120	20.0	0.0	0.0	1.00
Gravitational Force		1	0.94	0.92	0.66	0.15	0.10					
(range)			1.12	1.11	1.56	2.28	2.43					

of velocity, or acceleration, could be determined. The results of this procedure are given in Table 1.

The number of animals to orient upwards or downwards at six treatments of each of the four angles are listed in Table 1.

In order to compare the number of snails traveling upward at any particular angle and oscillation frequency, a χ-square test for homogeneity was used. The values of χ-square for different angles, together with the associated probability based on the assumption of homogeneity, are also presented in Table 1.

Thus for any given angle the associated probability indicates that a value of χ-square as large or larger than that observed would be achieved if the number of animals traveling upward was independent of oscillation frequency. For example, at 2° inclination, a value of χ-square greater than or equal to 1.32 would be observed with a probability of 0.93. Such a highly probable event does not constitute evidence in favor of a difference due to the rate of the mechanism or its resulting intermittent accelerations, for in only 7 of 100 trials would we expect to observe a smaller value of χ-square. It is concluded that the orientations observed were independent of the oscillations and intermittent accelerations provided by the apparatus in this experiment. The data on the mean, range, and standard deviation of the number of animals to orient upward at the different angles indicate progressive sensitivity to the angle of inclination (Figure 2). Note that the snails are very sensitive to the different angles of tilt, but do not exhibit a sensitivity to the intermittent nature of the gravity stimulation caused by the oscillating platform (Table 1).

Results indicate that in the cases observed an intermittent stimulus of gravity does not change the proportion of test animals that orient upward at any particular angle. In this study we attempted to approximate the conditions of Talbot's Law for reactions to light, using instead of light the acceleration of gravity. The experimental animals were subjected to five different conditions in which the reciprocal changes in intensity and time were unique. If a principle analogous to Talbot's Law could be applied to these conditions, using an entirely different stimulus parameter, the results obtained from a particular treatment should not differ significantly from any other treatment, or absence of treatment. According to the statistical procedures employed, the proportion of upward orientation at any particular angle is characteristic and the intermittent accelerations delivered do not change this proportion. Within a particular angle, the observed variation of proportions of animals orienting upward during

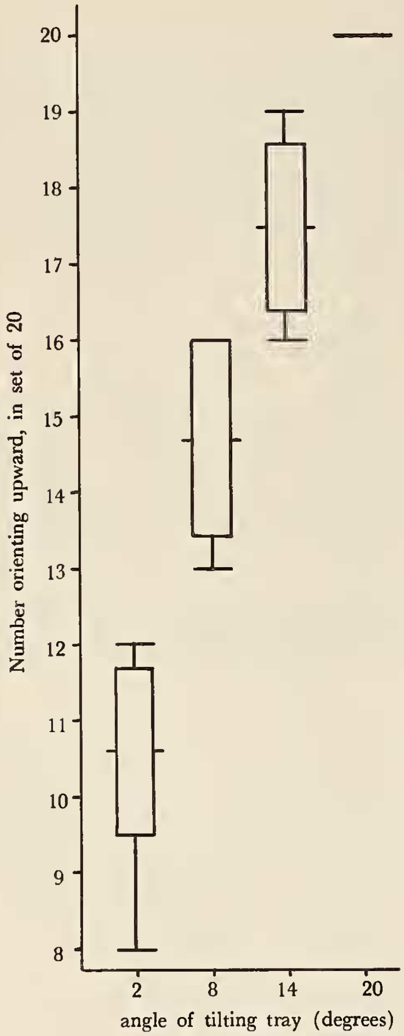

Figure 2:

Range, Mean, and Standard Deviation of Animals to Orient Upward in a Set of 20 at each Experimental Angle

the control and experimental conditions could be expected in the absence of treatment in at least 82% of the cases.

It is proposed that the existence of a general intermittent stimulus hypothesis should be investigated, using the principles heretofore found to govern animal reactions to light.

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SUMMARY

1. The effects of an intermittent stimulus of gravity on the geotactic behavior of the land snail *Helix aspersa* Müller have been investigated.
2. Animals were subjected to a vertical oscillation, through a distance of 69 cm at rates of 9.5, 14.5, 26, 41, and 54 oscillations per minute, with extremes of 0.1 and 2.4 gravities.
3. Geotactic behavior was measured by counting the proportions of animals to orient upward at angles of 2°, 8°, 14°, and 20°. Each of these angles was treated with 5 rates of oscillation and no oscillation, and the results compared.
4. Results indicate that the behavior at each angle is distinct, with 99% confidence, and that variations in rate of oscillation have no effect on this distinction. Statistical treatment of proportions of animals to orient upwards at each particular treatment indicate that with high probability (0.82) the same proportions could be obtained without treatment.
5. It is concluded that these snails are not differentially responsive to accelerative stimuli of the magnitude and time pattern applied, and a phenomenon analogous to that described by Talbot's Law of light reactions is operative for gravitational reactions as well. Talbot's Law of light reactions states that with intermittent stimulation, the effect will remain constant if the total amount of light is constant, and reciprocal variations of intensity and time will elicit constant effects.

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Rheotactic Responses in the Marine Mollusk *Littorina planaxis* Philippi

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(4 Text figures)

INTRODUCTION

The following experiments were carried out at Hopkins Marine Station of Stanford University in 1964 during April and May, the mating season of *Littorina planaxis* (Philippi, 1847). At the end of May a population survey showed that this species was found primarily between +4 feet and +10 feet from zero tide level. The *L. planaxis* used for the experiment were taken from two populations, which were between +3 feet and +8 feet.

Most of the *Littorina planaxis* at China Point, Monterey, California, range between 6 mm and 15 mm in height. The animals used in this study were between 9.5 mm and 13.5 mm. This size is slightly larger than the littorines in the intermediate group with which North (1954) worked in his maximum current study. North (*l. c.*) reports that a current of 230 cm/sec was sufficient to dislodge a majority of snails. The currents used in the present experiments ranged from 5 cm/sec to 58 cm/sec. The temperatures of the water used for the experimental currents varied from 12.6° C to 13.2° C, which is slightly warmer than the range of the morning sea temperatures for the same period.

A POSITIVE RHEOTAXIS

The first experiment (experiment A) was performed at each of the four tidal periods. The following procedure was used: (1) A pair of snails taken from the field was allowed to attach to a glass plate; a current initially aimed at the posterior end of the shell was then introduced and the behavior recorded for about 20 minutes; (2) the same pair was placed in a glass tube, 150 cm long and 2 cm in diameter; the tube was fixed horizontally on a black background and completely filled with water, which flowed at 27 cm/sec; the horizontal and angular movement of the pair was recorded for about 30 minutes; (3) step one was then repeated, and the pair of snails was stored in the laboratory in dry collection bottles. The apparatus was cleaned after each test to prevent old mucous trails from having any effect.

Sixteen animals were tested in this manner for two consecutive tidal periods. When snails were responding